Patent Application

for

SYSTEM AND METHOD FOR MANIPULATING MAGNETIC PARTICLES IN FLUID SAMPLES TO COLLECT DNA OR RNA FROM A SAMPLE

BACKGROUND OF THE INVENTION

Field of the Invention:

The present invention relates to a system and method for manipulating magnetic particles in a fluid sample to efficiently and effectively collect DNA or RNA that has been bound to the particles. More particularly, the present invention relates to a system and method employing movable magnets for holding and releasing magnetic particles in a fluid sample so that DNA or RNA bound to the magnetic particles can be separated from the fluid sample.

Description of the Related Art:

A variety of molecular biology methodologies, such as nucleic acid sequencing, direct detection of particular nucleic acids sequences by nucleic acid hybridization, and nucleic acid sequence amplification techniques, require that the nucleic acids (DNA or RNA) be separated from the remaining cellular components. This process generally includes the steps of collecting the cells in a sample tube and lysing the cells with heat and reagent which causes the cells to burst and release the nucleic acids (DNA or RNA) into the solution in the tube. The tube is then placed in a centrifuge, and the sample is spun down so that the various components of the cells are separated into density layers within the tube. The layer of the nucleic acids can be removed from the sample by a pipette or any suitable instrument. The samples can then be washed and treated with appropriate reagents, such as fluorescein probes, so that the nucleic acids can be

detected in an apparatus such as the BDProbeTec® ET system, manufactured by Becton Dickinson and Company and described in U.S. Patent No. 6,043,880 to Andrews et al., the entire contents of which is incorporated herein by reference. Although the existing techniques for separating nucleic acids from cell samples may be generally suitable, such methods are typically time consuming and complex. Furthermore, although the centrifuging process is generally effective in separating the nucleic acids from the other cell components, certain impurities having the same or similar density as the nucleic acids can also be collected in the nucleic acid layer, and must be removed from the cell sample with the nucleic acids.

A technique has recently been developed which is capable of more effectively separating nucleic acids from the remaining components of cells. This technique involves the use of paramagnetic particles, and is described in U.S. Patent No. 5,973,138 to Mathew P. Collis, the entire contents of which is incorporated herein by reference.

In this technique, paramagnetic particles are placed in an acidic solution along with cell samples. When the cell samples are lysed to release the nucleic acids, the nucleic acids are reversibly bound to the paramagnetic particles. The magnetic particles can then be separated from the remainder of the solution by known techniques such as centrifugation, filtering or magnetic force. The magnetic particle to which the nucleic acids are bound can then be removed from the solution and placed in an appropriate buffer solution, which causes the nucleic acids to become unbound from the magnetic particles. The magnetic particles can then be separated from the nucleic acids by any of the techniques described above.

Examples of systems and method for manipulating magnetic particles are described in U.S. Patent Nos. 3,988,240, 4,895,650, 4,936,687, 5,681,478, 5,804,067 and 5,567,326, in European Patent Application No. EP905520A1, and in published PCT Application WO 96/09550, the entire contents of each of said documents being incorporated herein by reference.

Although the paramagnetic particle technique is very effective in separating and harvesting nucleic acids from cell samples, a need exists for an improved technique for

manipulating the paramagnetic particles to provide an even more effective method of separation.

SUMMARY OF THE INVENTION

An object of the present invention is to provide an improved system and method for manipulating paramagnetic particles to which nucleic acid molecules are bound in a solution to effectively separate the nucleic acid molecules from the remaining components of the solution.

A further object of the present invention is to provide a system and method that is capable of altering the temperature of a cell solution to perform a lysing technique which enables nucleic acid molecules to become bound to paramagnetic particles in the solution, as well as being capable of manipulating the paramagnetic particles to appropriately separate the nucleic acid molecules from the remaining components of the solution.

A further object of the present invention is to provide a system and method for use in a nucleic acid assay preparation system, that is capable of heating and cooling sample solutions as appropriate to perform a lysing technique, and which is further capable of manipulating paramagnetic particles to which nucleic acid molecules of the lysed cell samples become bound, so that the assay preparation system can properly wash the nucleic acid molecules and place the nucleic acid molecules in a sample assay.

These and other objects are substantially achieved by providing a system and method for manipulating nucleic acid molecule-bound paramagnetic particles in a sample solution to separate the molecules from the remaining components in the solution. The system and method includes a tube receiver for receiving at least one sample tube containing a cell solution, paramagnetic particles such as iron oxide particles, and an acidic solution. The tube receiver is adapted for use with a system for preparing nucleic acid assays. The tube receiver includes a heating and cooling unit, such as a thermoelectric element, which is capable of heating the cell solution to lyse the cell and enable the nucleic acid molecules to become bound to the paramagnetic particles. The thermoelectric elements can also be used to quickly cool the solution as

necessary. The tube receiver further includes movable magnets which can be moved proximate to the outer wall of the tubes to attract the molecule-bound paramagnetic particle to the sides of the tubes, while the assay preparation system removes the remainder of the cell solution and washes the particles. The movable magnets can then be moved away from the tubes so that the molecule-bound paramagnetic particles are released from the walls of the tubes, so that the assay preparation system can eject an elution reagent, such as a suitable buffer solution, which causes the nucleic acid molecules to become unbound from the paramagnetic particles. The tube receiver further includes electromagnets which are activated to provide a magnetic field to the tubes to degauss the paramagnetic particles to allow the paramagnetic particles to mix with the elution reagent. The movable magnets can then be moved proximate to the sample tubes to adhere the paramagnetic particles to the walls of the sample tubes while the assay preparation system aspirates the nucleic acid molecules from the sample tubes. The assay preparation system can then place the nucleic acid molecules in the appropriate microtiter trays for reading by an assay reading system.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other objects, advantages and novel features of the invention will be more readily appreciated from the following detailed description when read in conjunction with the accompanying drawings, in which:

Fig. 1 is a diagram of an example of a nucleic acid assay preparation system employing a nucleic acid molecule extractor according to an embodiment of the present invention;

Fig. 2 is a perspective view of the nucleic acid molecule extractor shown in Fig. 1;

Fig. 3 is a top view of the nucleic acid molecule extractor shown in Fig. 2;

Fig. 4 is a exploded perspective view of an example of a tube rack used with the nucleic acid molecule extractor shown in Figs. 1-3;

Fig. 5 is a detailed view of an example of the shape of one of the openings in the tube rack shown in Fig. 4;

Fig. 6 is a cross-sectional view of the nucleic acid molecule extractor taken along lines 6-6 in Fig. 3;

Fig. 7 is a detailed view of the portion of the nucleic acid molecule extractor designated in Fig. 6;

Fig. 8 is a exploded perspective view showing an example of the relationship between the tube blocks, electromagnets and thermoelectric devices included in the nucleic acid molecule extractor shown in Figs. 1-3, 6 and 7;

Fig. 9 is a side view of the electromagnet printed circuit board shown in Fig. 8;

Fig. 10 is diagrammatic view illustrating the relationship of the fixed side and sliding cam of the nucleic acid molecule extractor shown in Figs. 1-3, 6 and 7 when the movable magnets are positioned as shown in Figs. 6 and 7;

Fig. 11 is a diagrammatic view illustrating the relationship between the fixed side and sliding cam of the nucleic acid molecule extractor shown in Figs. 1-3, 6 and 7 when the magnets are being moved in a downward direction away from the tubes; and

Fig. 12 is a diagrammatic view illustrating the relationship between the fixed side and sliding cam of the nucleic acid module extractor shown in Figs. 1-3, 6 and 7 when the movable magnets are positioned at the downward most position away from the tubes.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Fig. 1 illustrates a sample assay preparation system 100 for which a nucleic acid molecule extractor 102 is adapted for use. The system 100 includes a robot 104, such as a robot manufactured by Adept Corp. of San Jose, California, or any other suitable robot. The robot includes a pipette holding mechanism 106, which can releasably couple to a plurality of pipette tips (not shown) stored in pipette tip racks 108. The robot 104 further includes a suction mechanism (not shown) that can be activated to create a vacuum in tubing 110 to draw fluid into the pipette tips, or to create pressure in tubing 110 to eject fluid from the pipette tips for reasons discussed in more detail below.

As further shown in Fig. 1, a plurality of sample input tubes 112 in a sample tube holder are positioned at a predetermined location with respect to the area of

movement of the robot 104. In addition, bulk reagent containers 114, which include different reagents as discussed in more detail below, and a plurality of microtiter trays 116 are located at predetermined position with respect to the robot 104.

Further details of the extractor 102 are shown in Figs. 2-9 as will now be discussed. The extractor 102 includes a removable rack 118 into which can be placed a plurality of tubes 120 containing paramagnetic particles such as those described in U.S. Patent No. 5,973,138 referenced above. The extractor 102 further includes fixed sides 122 and cam plates 124 which extend parallel or substantially parallel to fixed sides 122 as shown. The extractor further includes a stepper motor 126 connected to a lead screw 128 which is controlled by a controller (not shown) of the system 100 to slide the cam plates 124 with respect to the fixed sides 122 for reasons discussed in more detail below. As shown, in particular, in Fig. 3, the extractor 102 includes a home sensor 130 that is connected to the controller (not shown). The home sensor detects the position of a home flag 132 to indicate to the controller the position of the cam plates 124 with respect to the fixed sides 122 for reasons discussed below.

As discussed above, the extractor 102 includes and is adaptable for use with a rack 118, the details of which are shown with more specificity in Figs. 4 and 5. In particular, the rack 118 includes a bottom 134 and a top 136. The bottom 134 includes a plurality of legs 138, a handle 140 and a plurality of openings 142 therein. As shown in Fig. 5, the openings 142 include edges 144 which are configured to engage with projections 146 on the exterior of the tubes 120 to prevent the tubes 120 from rotating within the openings 142 when, for example, a cap (not shown) is being screwed onto a top of the tube 120.

As further shown in Fig. 4, the bottom 134 of rack 118 includes two openings, each having a press-in nut 148 inserted therein. Each nut receives the threaded portion of a captive thumb screw 150 which secures the top 136 of the rack 118 to the bottom 134 after the tubes 130 have been inserted into the opening 142. The top 136 abuts against a shoulder 152 which is positioned proximate to the tops of the tubes 120, and thus prevents the tubes 120 from falling out of the rack 118, or being inadvertently lifted

out of the rack by the pipette tips discussed above, when the robot 104 is adding or removing solution to and from the tubes 120.

Further details of the extractor 102 are shown in Figs. 6-9 as will now be described. As illustrated, the extractor 102 includes a plurality of heat sink blocks 154 disposed between the fixed sides 122 and thus, in the interior of the extractor 102. In this example, the extractor includes six heat sink blocks 154. The heat sink blocks are supported by a base plate 156 of the extractor 102 as shown, in particular, in Fig. 6. Each fixed side 122 includes a cam slot 158 which extends in a vertical or substantially vertical direction. The cam slots receive shoulder screws 160 (see Figs. 2 and 3) which pass through cam slots 162 (see Fig. 2) and to respective cam slots 158. As described in more detail below, each pair of shoulder screws 160 (two aligned shoulder screws on opposite sides of the extraction 102) are coupled to a respective magnet carrier 164 to which is mounted a permanent magnet 166. In this example, the extractor 102 includes seven pairs of shoulder screws 160 and seven corresponding magnet carriers 164 and magnets 166. As discussed in more detail below, when the stepper motor 126 which is connected to the motor mount 125 and the cam plates 124, moves the cam plates 124 in a horizontal or substantially horizontal direction with respect to the fixed sides 122, the cam slots 162 force the shoulder screws 160 to move in a vertical direction along the fixed cam slots 158 and therefore raise or lower the magnet carriers 164 and their respective magnets 166 for reasons discussed below.

As further illustrated in Figs. 6 and 7, a thermoelectric device 168 is mounted to the top of each of the respective heat sink blocks 154. A respective tube block 170 is positioned on the top of each of the thermoelectric devices 168 as illustrated.

As further shown in Figs. 8 and 9, each respective tube block 170 includes a plurality of openings 172, which are each adapted to receive a respective tube 120. Also, in this example, three thermoelectric devices 168 are associated with each tube block 170 and therefore, three thermoelectric devices are mounted on the top of each respective heat sink block 154. The thermoelectric devices 168 can be controlled to apply heat to tube block 170 or to extract heat from tube 170, as can be appreciated by one skilled in the art, under the control of the controller (not shown). Each tube block

170 also has a resistive temperature device (RTD) sensor 174 for sensing the temperature of the tube block and providing a signal to the controller so that the controller can appropriately control the thermoelectric devices 168.

As further illustrated, each tube block 170 has a slotted opening 176 into which is received an electromagnet circuit board 178 having a plurality of electromagnets 180 mounted thereon. The electromagnets 180 each include a preform coil 182 surrounding an electromagnetic core 184, and are coupled in series to PCB traces 186, which are coupled via connection pads 188 to the controller (not shown). As discussed in more detail below, the controller applies a current to electromagnets 180 which causes the electromagnets to generate an alternating current (AC) magnetic field.

As further shown in Figs. 6 and 7, the adjacent tube blocks 170 are spaced at a sufficient distance to allow magnet carriers 164 and permanent magnets 166 to slide proximate to the tube openings 172 and therefore proximate to the tubes 120 for purposes discussed in more detail below. In this example, each tube block 170 includes tube rows, each having eight openings 172. The extractor 102 includes six tube blocks 170. Thus, the extractor 102 includes 96 openings 172.

The operation of the extractor 102 with respect to the system 100 will now be described with reference to Figs. 1-3, 6, 7 and 10-12. Initially, samples containing cells are provided in sample input tubes 112. These samples may be of any type, including biological fluids such as blood, urine and cerebrospinal fluid, tissue homogenates and environmental samples, that are to be assayed for nucleic acids (DNA or RNA) of interest. The robot 104 is first controlled to move to the pipette tip racks 108 to pick up a plurality of pipette tips, for example, four pipette tips (not shown). The robot 104 is then controlled to position the pipette tips over a respective number of sample tubes 112 and draw the samples into the respective pipette tips. The robot then moves the pipette tips over to the extractor 102, and releases the samples into respective sample tubes 120 that have been loaded in advance into the rack 118 positioned on the extractor 102.

Each sample tube 120 has been previously supplied with paramagnetic particles. Although any type of paramagnetic particle may be used, including particles having polymeric coatings, the particles disclosed in U.S. Patent No. 5,973,138 referenced

above are preferred. Each of the sample tubes 112 also has lyse solution which lyses the cell samples.

The above process continues until all of the samples from the sample input tubes 112 have been inserted into the corresponding tubes 120 in the extractor 102. It is noted that the number of samples drawn at each time (i.e., four samples in this example) can vary as desired. It is also noted that each time the robot draws its samples from sample tubes 112 into pipette tips and then dispenses those samples into corresponding tubes 120, the robot moves to a discard position to discard the pipette tips. The robot 104 then selects four new pipette tips to transfer four new samples from the input tubes 112 to the tubes 120.

Once all of the samples have been loaded into the respective sample tubes 120, the controller controls the thermoelectric devices 168 to apply heat to the solutions in the tube 120 to lyse the samples. Once the lysing has been completed, the controller controls the thermoelectric device 168 to extract heat from the tube blocks 170, the sampling tubes 120 and the solutions contained therein, to cool the solutions to substantially room temperature.

Once the lysing and cooling processes are completed, the robot 104 is controlled to transfer a suitable acidic solution, such as that described in U.S. Patent No. 5,973,138, into the sample tubes 120. To do this, the robot 104 moves back and forth between the pipette tip racks 108, the bulk reagent containers 114, extractor 102, and the pipette disposal section (not shown) to transfer the acidic solution to, for example, four tubes 120 at a time. The robot 104 transfers acidic solution to four corresponding tubes 120 and mixes the solution in the tubes 120 by drawing the solution into the pipette tips and discharging the solution back into the tubes 120 in a controlled manner, while raising and lowering the pipette tips into and out of the tubes 120 in a controlled manner to maintain minimum tip submersion.

Also at this time, the controller controls the electromagnets 178 to generate an AC magnetic field, which demagnetizes the particles 190 so that the particles can freely mix with the acidic solution. Once the robot 104 has transferred acidic solution to four corresponding tubes 120 and has performed the mixing operations, the controller turns

off the electromagnets to remove the AC magnetic field. The acidic solution that has been added to the cell sampling tube 120 causes the nucleic acid molecules to become bound to the paramagnetic particles 190. Once the acidic solutions have been added to the samples in the sample tubes 120, the controller controls the stepper motor 126 to move the cam plates 124 in a direction indicated by arrow A in Fig. 10. This drives the shoulder screw 160 in an upward direction along fixed cam slots 158 so that the magnets 164 are positioned proximate to the tubes 120. Therefore, the molecule-bound particles 190 become adherent to the sides of the tubes 120 as shown, for example, in Fig. 7.

The robot 104 is then controlled to use the pipette tips to remove the solution from the tubes 120 and discard the solution in a waste container (not shown). As in the operations discussed above, each time the robot 104 uses pipette tips to remove solution from respective tubes 120, the robot 104 discards the pipette tips and uses new pipette tips before repeating the process on the remaining tubes 120.

The robot 104 is then controlled to add a washing solution to each of the tubes 120. When the wash solution is being added to the tubes 120, the controller controls the cam plates 124 to move in the direction indicated by arrow B in Figs. 11 and 12, which causes the shoulder screws 160 to drive the magnet carriers 164 and, hence the permanent magnets 166, in a downward direction in their respective fixed cam slots 158. When the magnets 166 are moved away from the tubes 120, the particles 190 are allowed to fall back into the bottoms of the tubes 120. At this time, the controller controls the electromagnets 178 to generate an AC magnetic field, which demagnetizes the particles 190 so that the particles can freely mix with the wash solution being added to the tubes 120. A rapid sequence of 5 aspirate and dispense cycles is used to perform the mix the particles with the wash solution. Once the robot 104 has completed mixing the wash solution, the controller turns off the electromagnets to remove the AC magnetic field.

After the wash solution has been added and mixed with the particles, the controller controls the stepper motor 126 to move the cam plates 124 in the direction along arrow A shown in Fig. 10, to drive the magnets 166 in the upward direction to be proximate to the tubes 120. The magnets 166 thus secure the molecule-bound particles

190 to the sides of the tube again as shown in Fig. 7. The robot 104 is then controlled to use the pipette tips (not shown) to remove the wash solution from the tubes 120. This wash step may be repeated as many times as necessary to wash the particles, e.g., two times.

The robot 104 is then controlled to add an elution reagent, such as those described in U.S. Patent No. 5,973,138 referenced above, to the tubes 120. The elution solution causes the molecules to become unbound from the particles 190. In a manner similar to that described above, the robot 104 uses new pipette tips for each group of tubes 120 to which the elution solution is being added from the bulk reagent tank 114.

After the elution solution has been added to and mixed within all of the tubes 120, the stepper motor 126 is controlled to move the cam plates 124 along direction A, as shown in Fig. 10, to move the magnets 166 proximate to the tubes 120. The robot 104 is then controlled to use the pipette tips to transfer the elution solution containing the nucleic acid molecules that have been released from the particles 190 into the microtiter trays 116. As with the operations described, the robot 104 uses fresh groups of pipette tips to transfer each group of sample to the respective wells and the microtiter trays 116. Once all the samples have been transferred, the microtiter trays 116 can be placed in a suitable reading device, such as the BDProbeTec® ET system described above. In an alternative embodiment, microtiter trays 116 can be configured on a conveyer and conveyed automatically into the BDProbeTec® ET system.

Although only one embodiment of this invention has been described in detail above, those skilled in the art will readily appreciate that many modifications are possible in the exemplary embodiment without materially departing from the novel teachings and advantages of this invention. All such modifications are intended to be included within the scope of this invention as defined in the following claims.